Answer 1:

**Bibliographic Information**

**Preclinical studies of treosulfan demonstrate potent activity in Ewing's sarcoma.** Werner, Sebastian; Mendoza, Arnulfo; Hilger, Ralf A.; Erlacher, Miriam; Reichardt, Wilfried; Lissat, Andrej; Konanz, Claudia; Uhl, Marcus; Niemeyer, Charlotte M.; Khanna, Chand; Kontny, Udo. Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Albert-Ludwigs-University, Mathildenstr. 1, Freiburg, Germany. Cancer Chemotherapy and Pharmacology (2008), 62(1), 19-31. Publisher: Springer, CODEN: CCPHDZ ISSN: 0344-5704. Journal written in English. AN 2008:466828 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

**Abstract**

Objectives High-dose chemotherapy with the alkylating agent busulfan has been widely used in the treatment of patients with high-risk Ewing's sarcoma. Because of risks for toxicity, busulfan and radiotherapy can not be applied together, leading to the omission of one effective therapy component. Treosulfan is a deriv. of busulfan which has a lower side effect profile than busulfan and which can be used together with radiotherapy. We investigated the effect of treosulfan in a panel of Ewing's sarcoma cell lines on cell survival, cell cycle and apoptosis in vitro and compared it to busulfan. Furthermore, the anti-tumor effect of treosulfan was studied in an orthotopic Ewing's sarcoma mouse xenograft model. Methods Cell survival was measured by MTT assay and cell cycle anal. by flow cytometry. Apoptosis was analyzed via detection of DNA fragmentation, Hoechst 33258 staining, Annexin V, and cleavage of caspases-3 and 9. The effect of treosulfan and busulfan on primary tumor growth was assessed in Ewing's sarcoma xenografts in NOD/SCID mice (10 mice per group), pharmacokinetics of treosulfan were analyzed in nude mice. Results Treosulfan inhibited cell growth to at least 70% in all cell lines at concns. achievable in vivo. Treosulfan had a greater effect on the inhibition of cell growth at equiv. concns. compared to busulfan. The growth inhibitory effect of treosulfan at low concns. was mainly due to a G2 cell cycle arrest, whereas at higher concns. it was due to apoptosis. Apoptosis was induced at lower concns. compared to busulfan. In contrast to busulfan, treosulfan induced cell death in an apoptosis-deficient cell line at concns. achievable in vivo. In mice, treosulfan suppressed tumor growth at dosages of 2,500 and 3,000 mg/kg. Pharmacokinetic exposures of treosulfan in mice were similar to previous reports in human patients. At maximal tolerated dosages treosulfan had a higher anti-tumor activity than busulfan.

Conclusions Our results suggest that treosulfan has efficacy against Ewing's sarcoma cells in vitro and in mice. Therefore, controlled trials examg. the role of treosulfan in patients with Ewing's sarcoma are warranted.

Answer 2:

**Bibliographic Information**

**Application of chimerism-based drug-induced tolerance to rat into mouse xenotransplantation.** Tomita, Y.; Shimizu, I.; Iwai, T.; Zhang, Q.-W.; Okano, S.; Kajiwara, T.; Onzuka, T.; Tominaga, R. Cardiovascular Surgery, Kyushu University, Fukuoka, Japan. Scandinavian Journal of Immunology (2006), 64(4), 392-397. Publisher: Blackwell Publishing Ltd., CODEN: SJIMAX ISSN: 0300-9475. Journal written in English. CAN 146:182894 AN 2006:1199405 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

**Abstract**

The current crit. shortage of human donor organs has stimulated the feasibility of the xenogenic transplantation, such as swine to primate. We have previously reported the induction of donor-specific tolerance in MHC-disparated recipient mice by using our cyclophosphamide (CP)-induced tolerance conditioning. In this study, we examd. the efficacy of our CP-induced tolerance conditioning in xenogenic transplantation model. F344 rats and B10 mice were used as donors and recipients. Recipient mice were treated with donor spleen cells, CP, Busulfan and bone marrow cells, with or without prior NK-cell depletion. Donor mixed chimerism, and the presence of donor reactive T-cell population were analyzed by flow cytometry. The survival of the donor skin grafts were obsd. after the conditioning. Donor mixed chimerism was temporary induced but terminated at 10 wk after treatments. Donor-specific prolongation of the skin graft survival was obsd. after the treatments, however, grafts were rejected in the long term. NK-cell depletion, prior to the treatments, did not affect the levels of the mixed chimerism or graft prolongation. The donor-reactive recipient T-cell population was remained the same level as the untreated mice, suggesting the failure of the induction of the central T-cell

tolerance. Thus, partial efficacy of our CP-induced tolerance treatments in the rat to mice xenotransplantation was obsd. Our results suggested that the addnl. treatments were required to establish the stable xenogenic tolerance.

Answer 3:

**Bibliographic Information**

**Human cell engraftment after busulfan or irradiation conditioning of NOD/SCID mice.** Robert-Richard, Elodie; Ged, Cecile; Ortet, Jacqueline; Santarelli, Xavier; Lamrissi-Garcia, Isabelle; de Verneuil, Hubert; Mazurier, Frederic. INSERM E217, Universite V. Segalen, Bordeaux, Fr. Haematologica (2006), 91(10), 1384-1387. Publisher: Ferrata Storti Foundation, CODEN: HAEMAX ISSN: 0390-6078. Journal written in English. CAN 146:55137 AN 2006:1193017 CAPLUS 

**Abstract**

Human hematopoietic stem cell (HSC) xenotransplantation in NOD/SCID mice requires recipient conditioning, classically achieved by sublethal irradn. Pretreatment with immunosuppressive and alkylating agents has been reported, but has not been rigorously tested against std. irradn. protocols. Here, we report that treatment of mice with a single dose (35 mg/kg) of Busilvex, an injectable form of busulfan, enables equiv. engraftment compared to 3.5 Gy irradn. Mice treated with two doses of 25 mg/kg to reduce busulfan toxicity showed increased chimerism. Busulfan conditioning and irradn. resulted in comparable sensitivity of HSC detection as evaluated by limiting diln. anal.

Answer 4:

**Bibliographic Information**

**Antileukemic activity of treosulfan in xenografted human acute lymphoblastic leukemias (ALL).** Fichtner, I.; Becker, M.; Baumgart, J. Experimental Pharmacology, Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany. European Journal of Cancer (2003), 39(6), 801-807. Publisher: Elsevier Science Ltd., CODEN: EJCAEL ISSN: 0959-8049. Journal written in English. CAN 139:345400 AN 2003:206379 CAPLUS 

**Abstract**

Treosulfan (L-threitol-1,4-bis-methanesulfonate; Ovastat) is a bifunctional alkylating drug indicated for the treatment of advanced ovarian carcinoma. Recent data revealed immunosuppressive characteristics and substantial hematopoietic stem cell toxicity after repeated dosing of mice. Therefore, treosulfan is considered to be an alternative conditioning agent to busulfan (for example) administered prior to allogeneic/autologous stem cell transplantation of patients with hematol. malignancies. An antineoplastic activity for treosulfan has been previously shown in preclin. models of melanoma, breast, lung and renal-cell carcinomas. Here, in vivo antileukemic activity of treosulfan is compared with the activity of equitoxic doses of cyclophosphamide or busulfan for the first time using human acute lymphoblastic leukemia (ALL)-models of pediatric origin xenotransplanted into non-obese diabetic (NOD)/severe combined immunodeficient (SCID) mice. Treosulfan treatment achieved an optimum treated to control (T/C) value of 159% (survival time) against B-ALL-SCID 7 and a T/C value of 0% (tumor growth) against T-ALL-SCID 4 and proB-ALL-SCID 19, resp. Complete regression of established s.c. growing nodules of ALL-SCID 4 and 19 was obvious and long-term survivors without tumor re-growth were obsd. Equitoxic doses of busulfan (ALL-SCID 4, 7, 19) or cyclophosphamide (ALL-SCID 19) were less effective with regard to the nos. of complete regressions and the no. of cured animals. Side-effects included myelotoxicity and a small redn. in body wt., but these were tolerable. Treosulfan can be considered a highly active antileukemic drug whose corresponding clin. value is to be tested in appropriate protocols with leukemic patients.

Answer 5:

**Bibliographic Information**

**Characterization of the mechanisms of busulfan resistance in a human glioblastoma multiforme xenograft.** Hare, C. Bradley; Elion, Gertrude B.; Colvin, O. Michael; Ali-Osman, Francis; Griffith, Owen W.; Petros, William P.; Keir, Stephen; Marcelli, Susan L.; Bigner, Darell D.; Friedman, Henry S. Medical Center, Duke University, Durham, NC, USA. Cancer Chemotherapy and Pharmacology (1997), 40(5), 409-414. Publisher: Springer, CODEN: CCPHDZ ISSN: 0344-5704. Journal written in English. CAN 127:214766 AN 1997:548814 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

**Abstract**

Busulfan is an alkylating agent commonly used in the treatment of chronic myelogenous leukemia and in combination with cyclophosphamide in prepn. for allogeneic bone marrow transplantation. Serial treatment of a childhood high-grade glioma xenograft (D-456 MG) with busulfan resulted in a busulfan-resistant xenograft, D-456 MG(BR). Cross-resistance to 1,3-bis(2-chloroethyl)-1-nitrosourea was seen but not resistance to cyclophosphamide or CPT-11. Cytoplasmic levels of glutathione in D-456 MG(BR) were approx. one-half those found in D-456 MG. This depletion could not be explained by levels of glutathione-S-transferase, or by amplification, rearrangement, or increased levels of transcript of γ-glutamylcysteine synthetase. Furthermore, depletion of glutathione in D-456 MG did not alter busulfan activity. Quantitation of busulfan levels in D-456 MG and D-456 MG(BR) xenografts following treatment of mice at the dose lethal to 10% of the animals demonstrated that lower levels of drug were achieved in D-456 MG(BR). These studies suggest that alterations in drug transport or metab. of busulfan may play a role in the resistance of D-456 MG(BR) to this alkylator.

Answer 6:

**Bibliographic Information**

**Busulfan therapy of central nervous system xenografts in athymic mice.** Aaron, Rosemary H.; Elion, Gertrude B.; Colvin, O. Michael; Graham, Michael; Keir, Steven; Bigner, Darell D.; Friedman, Henry S. Department Pediatrics, Duke University Medical Center, Durham, NC, USA. Cancer Chemotherapy and Pharmacology (1994), 35(2), 127-31. CODEN: CCPHDZ ISSN: 0344-5704. Journal written in English. CAN 122:305997 AN 1995:535830 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

**Abstract**

We evaluated the antitumor activity of busulfan against a panel of tumor cell lines and xenografts in athymic nude mice derived from childhood high-grade glioma, adult high-grade glioma, ependymoma, and medulloblastoma. Busulfan displayed similar activity against a panel of four medulloblastoma cell lines (D283 Med, Daoy, D341 Med, and D425 Med) and four corresponding sublines with lab.-generated or clin. acquired resistance to 4-hydroperoxycyclophosphamide [D283 Med (4-HCR), Daoy (4-HCR), D341 Med (4-HCR), and D458 Med] and cross-resistance to melphalan. This is consistent with a nearly total lack of cross-resistance of busulfan to 4-hydroperoxycyclophosphamide. Busulfan was active in the therapy of all but one of the s.c. xenografts tested, with growth delays ranging from 14.3 days in D612 EP to 58.4 days in D528 EP. Busulfan produced statistically significant increases in the median survival of mice bearing intracranial D456 MG (66%-90%), D612 EP (18%-33%), and D528 EP (89%) xenografts. These studies suggest that busulfan may be active against medulloblastomas, high-grade gliomas, and ependymomas as well as against cyclophosphamide-resistant neoplasms.

Answer 7:

**Bibliographic Information**

**Busulphan is active against neuroblastoma and medulloblastoma xenografts in athymic mice at clinically achievable plasma drug concentrations**. Boland I; Vassal G; Morizet J; Terrier-Lacombe M J; Valteau-Couanet D; Kalifa C; Hartmann O; Gouyette A Laboratory of Pharmacotoxicology and Pharmacogenetics (CNRS URA147), Villejuif, France British journal of cancer (1999), 79(5-6), 787-92. Journal code: 0370635. ISSN:0007-0920. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, NON-U.S. GOV'T) written in English. PubMed ID 10070870 AN 1999168418 MEDLINE (Copyright (C) 2008 U.S. National Library of Medicine on SciFinder (R))

**Abstract**

High-dose busulphan-containing chemotherapy regimens have shown high response rates in children with relapsed or refractory neuroblastoma, Ewing's sarcoma and medulloblastoma. However, the anti-tumour activity of busulfan as a single agent remains to be defined, and this was evaluated in athymic mice bearing advanced stage subcutaneous paediatric solid tumour xenografts. Because busulphan is highly insoluble in water, the use of several vehicles for enteral and parenteral administration was first investigated in terms of pharmacokinetics and toxicity. The highest bioavailability was obtained with busulphan in DMSO administered i.p. When busulphan was suspended in carboxymethylcellulose and given orally or i.p., the bioavailability was poor. Then, in the therapeutic experiments, busulphan in DMSO was administered i.p. on days 0 and 4. At the maximum tolerated total dose (50 mg kg(-1)), busulphan induced a significant tumour growth delay, ranging from 12 to 34 days in the three neuroblastomas evaluated and in one out of three medulloblastomas. At a dose level above the maximum tolerated dose, busulphan induced complete and partial tumour regressions. Busulphan was inactive in a peripheral primitive neuroectodermal tumour (PNET) xenograft. When busulphan pharmacokinetics in mice and humans were considered, the estimated systemic exposure at the therapeutically active dose in mice (113 microg h ml(-1)) was close to the mean total systemic exposure in children receiving high-dose busulphan (102.4 microg h ml(-1)). In conclusion, busulphan displayed a significant anti-tumour activity in neuroblastoma and medulloblastoma xenografts at plasma drug concentrations which can be achieved clinically in children receiving high-dose busulphan-containing regimens.